MOLECULAR AND CELLULAR BIOLOGY, Jan. 1985, p. 99–108
0270-7306/85/010099-10$02.00/0

Vol. 5, No. 1

Characterization of Yeast Strains with Conditionally Expressed Variants of Ribosomal Protein Genes *tcm1* and *cyh2*

HOWARD M. FRIED,[1]* HONG GIL NAM,[2] STEVEN LOECHEL,[1] AND JOHN TEEM[3]†

Department of Biochemistry and Nutrition[1] and Department of Chemistry,[2] University of North Carolina, Chapel Hill, North Carolina 27514, and Department of Biology, Brandeis University, Waltham, Massachusetts 02254[3]

Received 16 July 1984/Accepted 24 October 1984

We placed a regulatory sequence derived from the *GAL10* locus of *Saccharomyces cerevisiae* at various distances from the start sites of transcription of two yeast ribosomal protein genes, *tcm1* and *cyh2*. The hybrid ribosomal protein genes were transcribed at wild-type levels in the presence of galactose. In the absence of galactose, the hybrid genes were transcribed either at a reduced level or essentially not at all. Yeast cells which transcribe the ribosomal protein genes at a reduced rate continued to grow, suggesting that enhanced translation of the ribosomal protein mRNA may permit an adequate rate of synthesis of the corresponding protein. Consistent with this suggestion is the finding that preexisting mRNA decayed at a reduced rate when transcription was halted abruptly by removal of galactose. Yeast cells unable to transcribe *tcm1* or *cyh2* without galactose did not grow. These conditional lethal strains demonstrate that the ribosomal proteins encoded by *tcm1* and *cyh2* are essential; furthermore, these strains are potentially useful for isolating mutations in the *tcm1* and *cyh2* proteins affecting their transport, assembly, or function.

Eucaryotic cells depend on the biogenesis of numerous complex multimeric structures for growth. Histones and other nuclear proteins organize the genome and facilitate its expression and replication. Actins, tubulins, and a variety of other filamentous proteins polymerize to construct the internal framework of the cell and to produce form and movement. Assembly of over 70 different ribosomal proteins about four rRNA molecules gives rise to the protein synthetic machinery of the cell. From a biochemical standpoint such complexes are interesting systems in which to study the relationship between macromolecular assembly and function. Moreover, there is ample evidence that synthesis of many of these housekeeping proteins is instrumental in regulating cell growth (18, 29). Thus, attention has also been focused upon identifying the mechanisms controlling the synthesis of these macromolecules.

In *Saccharomyces cerevisiae*, as in other eucaryotes, each of 70 or so ribosomal proteins is synthesized at the same relative rate. Immediately upon synthesis they are transported to the nucleolus, where they assemble at a rate which is sufficient to meet the ribosome requirements of a particular growth state yet which leaves no significant fraction of unassembled proteins. In addition, stimuli which alter the growth rate are manifested in immediate and simultaneous changes in the synthesis of all ribosomal proteins, to an extent characteristic of the new growth condition. Nonetheless, it is not known whether growth control of yeast ribosomal protein synthesis is regulated transcriptionally or posttranscriptionally. In addition, this highly coordinated and efficient production of ribosomal proteins occurs in a cell in which ribosomal protein genes are not closely linked but are distributed throughout the genome and in which some ribosomal protein genes occur in more than a single functional copy (see references 3 and 27 for reviews).

To investigate the controls which bring about the stoichiometric synthesis of yeast ribosomal proteins, we found it desirable to obtain conditionally expressed ribosomal protein genes, that is, ribosomal protein genes which are transcribed normally under one condition of growth but whose transcription is either reduced or eliminated under another condition. Ribosomal protein genes regulated in this fashion provided us with an opportunity to examine the consequences that selective deprivation of a single ribosomal protein or its mRNA has upon the coordinated synthesis of all the ribosomal constituents.

In this report, we describe the conversion of the yeast ribosomal protein genes *tcm1* and *cyh2* into derivatives which can be conditionally expressed. This conversion was achieved by joining each of the two ribosomal protein genes to a 365-base-pair (bp) activation sequence derived from the region separating the *GAL1* and *GAL10* genes of *S. cerevisiae* (24). The ribosomal protein genes then displayed the same behavior as *GAL1* and *GAL10*; that is, they required galactose and the *GAL4* regulatory protein for expression (8, 23). However, by placing the *GAL* sequence at various distances from the start of *tcm1* and *cyh2*, we obtained derivatives of the ribosomal protein genes which exhibited either low levels of expression or no expression in the absence of galactose or *GAL4*. A strain with a weakly expressed derivative of *tcm1* underproduced that ribosomal protein mRNA; nevertheless, the mRNA deficiency did not prevent growth, suggesting that an enhanced rate of translation of *tcm1* mRNA may operate to maintain synthesis of the ribosomal protein. Removal of galactose from a strain unable to transcribe *tcm1* in its absence caused preexisting *tcm1* mRNA to turn over more slowly than normal, a result consistent with enhanced translation. Finally, strains with derivatives of *tcm1* and *cyh2* which are completely dependent upon galactose for expression did not grow in its absence. Besides demonstrating the requirement of these two ribosomal proteins for viability, these strains serve as hosts for the isolation of mutants of the *tcm1* and *cyh2* proteins unable to complement the conditional growth phenotype. Such mutations may affect the transport, assembly, or function of these ribosomal proteins.

* Corresponding author.
† Present address: Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

MATERIALS AND METHODS

Bacterial and yeast strains. *Escherichia coli* strain MC1061 [*araD139* Δ(*ara-leu*)*7697* Δ*lacX74 galU galK hsr rpsL*] was provided by M. Casadaban and used as a host for all plasmids. *S. cerevisae* DBY745 (*MATα ura3-52 leu2-3 leu2-112 ade1-100*) was obtained from D. Botstein. HF193 (*MAT* **a**/α *ura3-52/ura3-52 ade1-100/ADE1 leu2-3 leu2-112/LEU2 his3/HIS3 his7/HIS7 aro2/ARO2 trp5/TRP5 lys5/LYS5 tcm1/TCM1*) is a diploid yeast strain heterozygous for resistance to trichodermin. HF194 (*MAT* **a**/α *ura3-52/ura3-52 ade1-100/ADE1 leu2-3 leu2-112/LEU2 his3Δ1/HIS3 trp1-289/TRP1 tyr1/TYR1 cyh2/CYH2*) is a diploid strain heterozygous for resistance to cycloheximide. Strain SJΔ1 (*MATa ura3-52 leu2-3 leu2-112 gal4 ΔLEU2*) was kindly provided by S. Johnston. This strain contains an insertion in the *GAL4* gene and produces no *GAL4* gene product.

Enzymes, chemicals and media. Restriction endonucleases, DNA polymerase I Klenow fragment, phage T4 DNA ligase, and other DNA-modifying enzymes were obtained from Bethesda Research Laboratories, Gaithersburg, Md. *O*-Nitrophenyl-β-galactoside, 5-bromo-4-chloro-3-indolyl-β-*D*-galactoside (X-GAL), and cycloheximide were purchased from Sigma Chemical Co., St. Louis, Mo. Trichodermin was the generous gift of W. O. Godtfredsen, Leo Pharmaceuticals, Ballerup, Denmark.

Plasmids were introduced into yeast cells essentially as described (7). Yeast transformants were grown in 2% glucose–0.7% yeast nitrogen base (Difco Laboratories, Detroit, Mich.) or 2% galactose–0.7% yeast nitrogen base and supplemented with 40 μg of required amino acids and adenine per ml. For indication of β-galactosidase in yeast cells, solid medium was buffered with 0.1 M $NaPO_4$ at pH 7 and contained 100 μg of X-GAL per ml. The requirement of the *GAL4* gene product for expression of the hybrid genes (see below) was demonstrated as follows: plasmids were introduced into yeast strain SJΔ1, and the resulting transformants were grown in 2% galactose–2% glycerol–2% lactic acid (pH 6)–0.7% yeast nitrogen base.

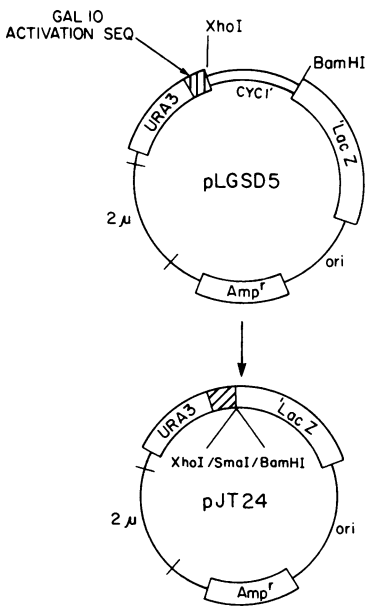


FIG. 1. Derivation and structure of generalized cloning vector for galactose-regulated transcription. Plasmid pJT24 was obtained by removal of the yeast *CYC1* (iso-1-cytochrome *c*) gene sequences from plasmid pLGSD5 (5), with concomitant insertion of oligonucleotides comprising recognition sites for the restriction enzymes shown (see the text). These sites separate the *GAL10* activation sequence (crosshatched box) from the *E. coli* β-galactosidase gene (*lacZ*). Yeast gene segments containing a promoter and adjacent transcribed sequences can be inserted between *GAL10* and *lacZ* to yield a hybrid transcription unit which, when introduced into yeast cells, is induced by the presence of galactose in the growth medium. The plasmid also contains origins of replication for *E. coli* (ori) and yeast (2 μ) and genes for selection of the plasmid in *E. coli* (Amp^r) and yeast (URA3).

Plasmid construction. Plasmid pLGSD5 was generously made available by L. Guarente. It contains the 365-bp *GAL10* activation sequence joined to the 5′ flanking sequence of the *CYC1* gene, which itself is joined to the *E. coli* β-galactosidase gene (*lacZ*) (5). Plasmid pJT24 (J. Teem, Ph.D. thesis, Brandeis University, Waltham, Mass., 1983) was constructed by digesting pLGSD5 with *Bam*HI, which cleaves at the junction of *CYC1* and *lacZ* (Fig. 1). A *Sma*I-*Bam*HI adapter oligonucleotide was ligated into the *Bam*HI site, and the plasmid was recircularized. The resulting plasmid was then digested with *Xho*I, which cleaves at the junction of *CYC1* and the *GAL10* activation sequence. The *Xho*I ends were filled in with DNA polymerase I and ligated to a similarly filled-in *Bam*HI-*Sma*I adapter. This regenerated the *Xho*I site on each side of the *Sma*I site, yielding *Xho*I-*Sma*I-*Xho*I where the single *Xho*I site had been previously. The resulting plasmid was then digested with *Sma*I, cleaving at the *GAL10-CYC1* junction and the *CYC1-lacZ* junction. Recircularization of the plasmid deleted all of the *CYC1* insert in pLGSD5, producing plasmid pJT24, which carries *GAL10* separated from *lacZ* by an *Xho*I-*Sma*I-*Bam*HI linker.

Plasmid pGTCM-Z is a derivative of pJT24 which contains a segment of *tcm1* inserted between the *GAL10* and *lacZ* sequences. It was produced by treating 1 μg of an *Hpa*I-*Bgl*II restriction enzyme fragment of *tcm1* (beginning 228 nucleotides upstream and ending 329 nucleotides downstream of the transcriptional start site of *tcm1*; reference 21; unpublished data; see Fig. 2) with 200 U of BAL 31 nuclease per ml. Aliquots of the nuclease digestion reaction were removed at 30-s intervals, extracted with phenol, and ligated to pJT4 that had been previously cleaved with *Sma*I and treated with *E. coli* alkaline phosphatase. The ligated DNA was introduced into MC1061; plasmid DNA was prepared from a pool of the resulting *E. coli* transformants and introduced into yeast strain DBY745. Yeast transformants were screened on glucose or galactose medium containing X-GAL. Plasmid extracts were prepared from yeast cells which hydrolyzed more X-GAL in the presence of galactose than in the presence of glucose, and the plasmids were recovered in *E. coli*. pGTCM-Z is one such plasmid. It was digested with *Xho*I and *Bam*HI cleaving on each side of the *tcm1* insert, and the insert was cloned into the single-stranded phage vectors M13mp8 and M13mp9 (14) for dideoxynucleotide sequence analysis. This analysis revealed that the *GAL10* sequence had become joined to −179 of *tcm1* and the *lacZ* segment had become joined to +313.

Plasmid pGTCM-Z2 was obtained by purifying an *Rsa*I restriction fragment of *tcm1* (from −194 to +43) and inserting it at the *Sma*I site of pJT24. Examination of the sequences of *tcm1* and *lacZ* revealed that the distal end of the *Rsa*I fragment joined to *lacZ* results in an in-frame fusion of the two translational reading frames. The orientations of this *Rsa*I fragment and all subsequent *tcm1* fragments (see below) in pJT24 were confirmed by nucleotide sequence analysis.

The *lacZ* moiety in pGTCM-Z was relocated from position +313 to +43 of *tcm1* as follows. The plasmid was digested to completion with *Sst*I, which cleaves within the *lacZ* moiety. The *Sst*I-cleaved pGTCM-Z was then digested partially with *Xba*I, which cleaves at position −1 in the adjacent *tcm1* moiety as well as in one other site in the plasmid. The incompletely digested fragment lacking only the *tcm1-lacZ Xba*I-*Sst*I segment was purified and joined to the *Xba*I-*Sst*I *tcm1-lacZ* segment derived from pGTCM-Z2. The result was plasmid pGTCM-Z1, containing *GAL10* at −179 and *lacZ* at +43 in *tcm1*.

Plasmid pGTCM-Z3 was obtained by inserting a *Sau*3A fragment of *tcm1* (from −138 to +136) into the *Bam*HI site of pJT24. The resultant plasmid was then subjected to the same *Xba*I-*Sst*I digest as described above for pGTCM-Z, followed by insertion of the *Xba*I-*Sst*I segment of pGTCM-Z2, thus placing *GAL10* at −138 and *lacZ* at +43 in *tcm1*. Plasmid pGTCM-Z4 was obtained by inserting an *Hpa*I-*Rsa*I fragment of *tcm1* (from −229 to +43) into the *Sma*I site of pJT24. The fragment was obtained by partial *Rsa*I digestion of the same *Hpa*I-*Bgl*II fragment used to create pGTCM-Z. Plasmid pGTCM-Z5 was produced by inserting a *Pvu*II-*Rsa*I fragment of *tcm1* (from −465 to +43) into the *Sma*I site of pJT24. This fragment was also the result of a partial *Rsa*I digest of the *Pvu*II-*Bgl*II fragment of *tcm1*. Plasmid pGTCM-Z6 was constructed by inserting a *Bst*NI-*Rsa*I fragment of *tcm1* (from −90 to +43) into the *Sma*I site of pJT24. The *Bst*NI end was rendered flush with DNA polymerase I before joining to pJT24.

Plasmid pGCYH is a derivative of pJT24 which contains a *Tha*I restriction enzyme segment of *cyh2* inserted at the *Sma*I site. This insertion placed the *GAL10* activation sequence at ca. −160 relative to the start site of transcription (*cyh2* has three start sites [11]). However, the *cyh2* coding sequence is not joined in frame with *lacZ*, as the distal end of the *Tha*I fragment lies about 800 bp beyond the transcription termination sites of *cyh2*. To join *lacZ* to the *cyh2* reading frame, pGCYH was cleaved at the *Bgl*II site in *cyh2* and at the *Bam*HI site in *lacZ*, treated with S1 nuclease, and religated. The resulting plasmid, pGCYH-Z1, contains *lacZ* joined in frame at amino acid no. 111 of *cyh2*.

Preparation of yeast RNA and blot hybridization analysis. RNA was extracted from yeast cells as described (28). Total cellular RNA was denatured in 50% formamide–2.2 M formaldehyde and separated by size in 1.8% agarose gels containing formaldehyde (26). Gels were blotted to nitrocellulose and hybridized to ^{32}P-labeled probes as described. The probes consisted of M13 phage derivatives containing sequences complementary to *tcm1* or *cyh2* mRNA. Single-stranded M13 phage DNA was prepared by the method of J. Griffith (personal communication) and rendered partly double stranded in the presence of 5′-[α-^{32}P]dATP by the method of Hu and Messing (9). Densitometric scans of RNA hybridization autoradiographs were performed at a Hoeffer GS300 densitometer coupled to an Appligration (Dynamic Solutions Corp., Pasadena, Calif.) integration computer system.

Cells transformed with plasmids pGTCMΔ3′A and pGTCMΔ3′B (see below) contain, in addition to a complete copy of the *tcm1* gene, a *tcm1* gene in which plasmid pBR322 has been inserted at position +43. To prevent detection of possible *tcm1*-pBR322 transcripts in these cells, a segment of *tcm1* DNA which contains only sequences distal to position +43 was cloned into M13 and used as a hybridization probe.

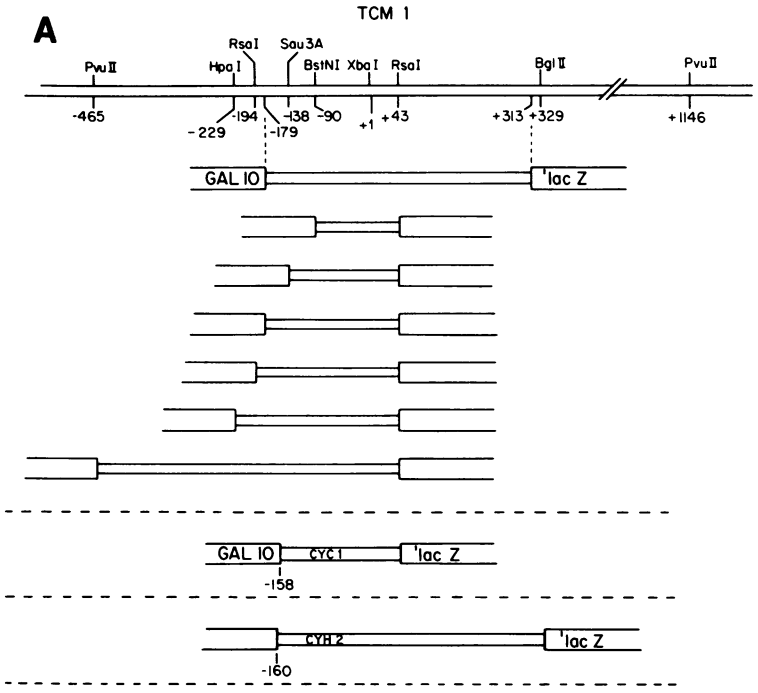
β-galactosidase assays. Yeast cells were grown to about 10^7 cells per ml, washed once, and resuspended in Z buffer (15). Cells were broken by vigorous shaking in the presence of glass beads, and the unclarified extracts were assayed for β-galactosidase activity by hydrolysis of *O*-nitrophenyl-β-D-galactoside (15).

RESULTS

Construction of a galactose-inducible ribosomal protein gene promoter. Our original aim was to convert a constitutively expressed ribosomal protein gene into one which could be induced by simple manipulation of the growth conditions. Our strategy was based upon the demonstration by Guarente and co-workers that substitution of a 365-bp DNA segment derived from upstream of the *GAL10* gene for the upstream region of the iso-1-cytochrome *c* gene (*CYC1*) conferred galactose inducibility upon cytochrome *c* (5). We anticipated that a similar substitution of this *GAL10* DNA segment into the upstream region of a ribosomal protein gene would render that gene galactose inducible as well. The experiments of Guarente et al. also showed that the *GAL10-CYC1* hybrid promoter was inactive in the presence of glucose, but they suggested that sequences outside of the *GAL10* segment were responsible for repression on glucose. Nonetheless, as we show in a later section, it is possible to mimic repression in the absence of galactose, provided a sufficient amount of upstream sequence of the ribosomal protein genes is removed and replaced by the *GAL10* sequence.

The activity of the *GAL10-CYC1* promoter was monitored by joining the *CYC1* coding sequence to *lacZ* (5). From the tripartite *GAL10-CYC1-lacZ* gene contained in plasmid pLGSD5, we constructed a generalized cloning vector to facilitate placing any gene under galactose regulation (see above). The vector, plasmid pJT24, consists of the 365-bp *GAL10* activation sequence followed immediately by the *lacZ* coding sequence (Teem, Ph.D. thesis) (Fig. 1). An oligonucleotide linker containing three unique restriction enzyme sites is interposed between *GAL10* and *lacZ*. The *GAL10* sequence is unable by itself to promote transcription, and the *lacZ* sequence lacks a translational initiation codon, so that plasmid pJT24 does not produce β-galactosidase. Insertion of a DNA segment containing a promoter and its adjacent coding sequence into one of the sites separating the *GAL10* and *lacZ* sequences in pJT24 should result in galactose-regulated expression of β-galactosidase, provided the transcribed sequence of the inserted DNA becomes fused in the same translational reading frame as *lacZ*. This expectation was verified as follows. A restriction enzyme segment of the ribosomal protein gene *tcm1*, extending from upstream of its transcriptional start site to within the coding sequence, was treated with nuclease BAL 31 and inserted between *GAL10* and *lacZ* in pJT24, producing a pool of plasmids with variously resected fragments of *tcm1* (see above). The plasmid pool was introduced into yeast cells, and transformants were selected which hydrolyzed more X-GAL in the presence of galactose than in the presence of glucose, suggesting that they contained a plasmid with a galactose-inducible β-galactosidase gene. Plasmids were then reisolated from yeast cells having the desired galactose-inducible X-GAL phenotype. One such plasmid, pGTCM-Z, was found by DNA sequence analysis to have the *GAL10* activation sequence at −179 relative to the *tcm1* transcriptional start site and the *lacZ* sequence joined in the correct reading frame at amino acid no. 95 of rpL3.

To determine the relative levels of expression of the *GAL10-tcm1-lacZ* gene in pGTCM-Z, we reintroduced the plasmid into yeast cells and performed β-galactosidase as-



B UNITS OF β-GALACTOSIDASE

	5′ ENDPOINT	GAL 4 GAL	GAL 4 DEX	gal 4 GAL + G/L	G/D
pGTCM-Z	-179	9.5	1.5	—	6.3
pGTCM-Z6	-90	680	0.5	0.5	1360
pGTCM-Z3	-138	622	0.7	5.0	888
pGTCM-Z1	-179	629	2.0	5.0	315
pGTCM-Z2	-194	664	2.5	10.0	265
pGTCM-Z4	-229	650	150	386	4.3
pGTCM-Z5	-465	533	384	580	1.4
pLGSD5	-158	420	0.5	—	840
pGCYH-Z1	-160	123	0.5	0.5	246

FIG. 2. Summary of the structure and activity of galactose-inducible yeast ribosomal protein-β-galactosidase genes. (A) Segments of various yeast genes which were placed between the *GAL10* activation sequence and the *lacZ* gene in plasmid pJT24. The topmost portion shows a restriction enzyme map of the *tcm1* gene, which encodes ribosomal protein L3. The numbers refer to the distance in base pairs from the start site of transcription of *tcm1*. Accordingly, plasmid pGTCM-Z contains the *GAL10* segment 179 nucleotides upstream of this start site and the *lacZ* gene 313 nucleotides downstream. Plasmids pGTCM-Z6, pGTCM-Z3, pGTCM-Z1, pGTCM-Z2, pGTCM-Z4, and pGTCM-Z5 all contain *lacZ* at position +43 of *tcm1* with *GAL10* located at −90, −138, −179, −194, − 229, and −465, respectively. Plasmid pLGSD5, depicted between the horizontal dashed lines, contains a segment of the yeast *CYC1* gene and was constructed by Guarante and co-workers (5). Plasmid pGCYH-Z1 carries a segment of the yeast *cyh2* gene, which encodes ribosomal protein L29. The *GAL10* segment is positioned at −160 relative to the start of transcription of *cyh2*. The *lacZ* segment is at +360 of *cyh2* (in terms of its mature transcript). Note that *cyh2* contains a 510-nucleotide intervening sequence which has been omitted from the diagram for clarity. (B) Results of β-galactosidase assays performed on extracts of yeast cells carrying the various plasmids. The cells were grown in medium with either 2% galactose (GAL), 2% glucose (DEX), or 2% galactose–2% glycerol–2% lactic acid (GAL + G/L) as carbon source. The column headed 5′ endpoint indicates the location of the *GAL10* activation sequence upstream of the start sites of the various genes. The column headed *GAL4* gives results obtained for plasmids carried in a yeast strain with a functional *GAL4* gene product. Conversely, the column headed *gal4* gives results from a yeast strain unable to produce this positive regulatory protein. The column headed G/D is the ratio of β-galactosidase activity in the *GAL4* strain grown on galactose and glucose.

says on transformants grown in galactose or glucose. Although the synthesis of β-galactosidase directed by pGTCM-Z was induced by galactose, the maximum level of expression was about 45 times lower than that exhibited by the *GAL10*-CYC1-*lacZ* gene in pLGSD5 (Fig. 2). We noticed, however, that cells containing pGTCM-Z grew on galactose at less than half the rate at which they grew on glucose and at half the rate of cells carrying pLGSD5 (data not shown); thus, we surmised that the ribosomal protein L3-β-GAL fusion protein is perhaps harmful to the cell. This hypothesis was verified by inserting into pJT24 a second fragment of *tcm1* whose upstream end is located at position −194, only 15 bp further from the site at which *tcm1* adjoins *GAL10* in pGTCM-Z but whose downstream end is located 43 bp instead of 313 bp from the amino terminus of rpL3 (see above). The hybrid polypeptide encoded by the new plasmid, pGTCM-Z2, consists of merely the first five amino acids of rpL3 fused to *lacZ* rather than the first 95. pGTCM-Z2 produced ca. 70 times more β-galactosidase than pGTCM-Z, and expression of the hybrid gene still required galactose (Fig. 2B). We also relocated the *lacZ* segment of pGTCM-Z to amino acid no. 5 with the result that β-galactosidase synthesis was likewise increased 70-fold (Fig. 2, pGTCM-Z1). Apparently, the original pGTCM-Z contained adequate *tcm1* promoter sequence for efficient expression, but its hybrid polypeptide product was either unstable, functionally impaired, or deleterious. (We have learned recently that the 95-amino acid fusion may impair cell viability by association with the nucleus of the cell. [L. Hereford and R. Moreland, unpublished data].) Thus, in confirmation of the work of Guarente and co-workers (5), we have shown that the *GAL10* activation sequence is an effective tool for converting any gene into a galactose-regulated derivative.

Effect of position of *GAL10* activation sequence in *tcm1*. As we mentioned above, the *GAL10* activation sequence is not believed to mediate repression of transcription by glucose (5). Yet the hybrid promoters described above were nearly totally inactive in the presence of this nutrient. Since ribosomal protein genes themselves are not subject to glucose repression, we constructed additional hybrid promoters to determine if the particular location of the *GAL10* sequence in *tcm1* was important for the observed expression. Placement of the *GAL10* sequence nearer the start site of transcription, at −138 in pGTCM-Z3 or at −90 in pGTCM-Z6, resulted in activity similar to pGTCM-Z1 and -Z2; that is, expression was high on galactose and nearly undetectable

on glucose (Fig. 2). Actually, pGTCM-Z3 and pGTCM-Z6 exhibited reproducibly less activity than pGTCM-Z1 or -Z2 on glucose. This difference in expression is in fact significant, as revealed by experiments described below in which the hybrid promoters were introduced into the yeast genome. Of further interest is the fact that only ca. 10 bp separate the *GAL10* sequence from the TATA homology of *tcm1* in pGTCM-Z6; nonetheless, transcription of the hybrid gene was as efficient as in those genes with more distance between these two elements.

Two other hybrid promoters we created contain *GAL10* at more distant locations from the start of *tcm1*. In pGTCM-Z4, the activation sequence resides at −229, which is only 34 bp further upstream in *tcm1* than in pGTCM-Z2. Surprisingly, this small change eliminated almost completely the distinction between the two carbon sources, there being only a factor of four difference between glucose and galactose (Fig. 2B). In pGTCM-Z5 we moved *GAL10* another 236 bp further upstream, and this promoter was completely unaffected by growth conditions.

These results show that the ability of the *GAL10* segment to perform as a regulatory element is affected strongly by its location within a relatively short span of *tcm1* 5′ flanking sequence. Assuming that the *GAL10* sequence acts only as an activator of transcription and that it does not mediate glucose repression, we suggest that the span of *tcm1* between about −229 and −138 is the sequence which normally activates transcription of this ribosomal protein gene. By placing the *GAL10* segment at −138, we simply deleted completely the *tcm1* activation sequence, replacing it with a second such sequence that relies on galactose for activity. When the *GAL10* segment is located at −229, its presence becomes irrelevant due to retention of the complete *tcm1* activation sequence, and thus, no distinction is seen in expression on the two carbon sources. Placement of the *GAL10* segment at a position intermediate between −229 and −138 leaves a portion of the *tcm1* activation sequence sufficient to give a low level of transcription when the *GAL10* segment is not activated. Indeed, we deleted the *GAL10* activation sequence from all of the plasmids described here, leaving only various extents of the 5′ flanking sequence of *tcm1* (manuscript in preparation). Retention of the *tcm1* sequence out to −229 yielded full transcriptional activity, whereas retention of sequence out to only −138 abolished activity completely. Deletions ending at points in between −229 and −138 retained an intermediate level of activity (data not shown). Note also that the hybrid promoters dependent upon the *GAL10* sequence for expression also required the *GAL4* regulatory protein (Fig. 2) which, as indicated above, is believed to interact with the *GAL10* activation sequence to promote transcription.

Construction of yeast strains with conditionally expressed *tcm1* genes. Having constructed hybrid *tcm1* promoters dependent upon galactose for expression, we wished next to substitute the inducible promoters for their wild-type constitutive counterpart in the yeast genome. Figure 3 illustrates the method used to accomplish this substitution. Plasmids pGTCM-Z2 and pGTCM-Z3 were digested with *Bam*HI and *Hin*dIII to produce a restriction fragment carrying the *GAL10-tcm1*-fused sequences but lacking the *lacZ* moiety. The

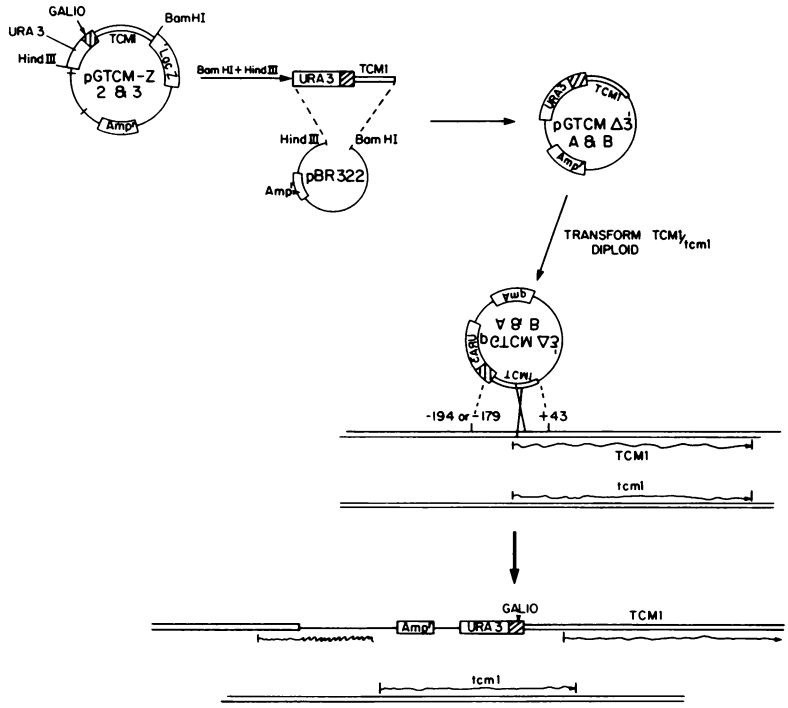


FIG. 3. Replacement of the promoter of *tcm1* in the yeast genome with *GAL10* regulated derivatives. A *Bam*HI-*Hin*dIII restriction fragment, carrying the *URA3* gene and the *GAL10-tcm1* fused sequences, was excised from plasmids pGTCM-Z2 and pGTCM-Z3 and inserted into plasmid pBR322. The resulting plasmids, pGTCMΔ3′A and pGTCMΔ3′B, contain the region of *tcm1* between −194 and +43 and the region between −138 and +43, respectively. These derivatives were introduced into a *TCM1/tcm1* diploid yeast strain, permitting recombination of the *tcm1* sequence in the plasmid with the homologous region in the yeast genome. One such recombination event, depicted at the bottom of the figure, creates duplicate *tcm1* promoters separated by the plasmid. One promoter is wild-type, but it directs transcription of an rpL3 coding sequence interrupted by plasmid sequence at position +43. The second promoter brings about transcription of an intact rpL3 coding sequence, but it is now subject to regulation by the *GAL10* activation sequence, positioned either at −194 or −138.

fragment also contains the *URA3* gene. The two *Bam*HI-*Hin*dIII fragments were joined to pBR322, yielding plasmids pGTCMΔ3′A and pGTCMΔ3′B which contain *GAL10* at −194 and −138, respectively, but lack all *tcm1* sequence beyond +43. Each plasmid was used to transform the diploid yeast strain HF193 to uracil prototrophy. Since neither plasmid is capable of self-replication in yeast cells, transformants are obtained only when the plasmid recombines with the host genome (7). Recombination of the plasmid at an rpL3 locus caused that gene to become interrupted by plasmid DNA sequence (Fig. 3). Simultaneously, the galactose-dependent *tcm1* promoter became linked to an intact rpL3 coding sequence. Thus, the recombinant chromosome in the transformed cell directs synthesis of rpL3 only in the presence of galactose. Note also that *tcm1* is a mutant allele of rpL3 which confers resistance to trichodermin, an inhibitor of protein synthesis (1, 4). Resistance is, however, semidominant; a *TCM1*/*tcm1* heterozygote (such as HF193) grows in 2 but not in 10 μg of trichodermin per ml. If for example pGTCMΔ3′ recombined with the *TCM1* allele in HF193, the transformant would become resistant to 10 μg of trichodermin per ml, provided glucose was present, since then only the *tcm1* allele would be expressed. On galactose, however, the same transformant would grow only in 2 μg of trichodermin per ml, since both *TCM1* and *tcm1* would be expressed. A reverse situation would apply if the plasmid recombined with the *tcm1* allele in the heterozygous diploid. Thus, a galactose-dependent change in resistance phenotype signals the desired recombination event (1).

A number of transformants were obtained whose resistance phenotype displayed a carbon source dependency. One transformant arising from each of the two plasmids was allowed to undergo meiosis and sporulation. All Ura$^+$ haploid progeny from the pGTCMΔ3′A transformant (*GAL10* at −194) grew normally on galactose but slower on glucose. Ura$^+$ haploids from the pGTCMΔ3′B transformant (*GAL10* at −138) failed to grow on glucose, whereas they grew normally on galactose. This analysis revealed that, indeed, the two plasmids had recombined with an rpL3 locus in HF193; the Ura$^+$ marker carried by the plasmid is linked to the growth defect on glucose, which itself is expected to be the result of reduced or absent transcriptional activity of the essential rpL3 gene, now under control of one of the two hybrid promoters. The structure of the recombinant chromosome in pGTCMΔ3′ transformants was also verified by Southern blot hybridization (data not shown). Furthermore, the slow-growth phenotype of the pGTCMΔ3′A derivative and the nongrowth phenotype of the pGTCMΔ3′B derivative coincide with the activities of the hybrid promoters they contain; the former promoter (in pGTCM-Z2) directed synthesis of a greatly reduced yet detectable amount of β-galactosidase on glucose, whereas the latter promoter (in pGTCM-Z3) produced virtually no detectable β-galactosidase on the same carbon source.

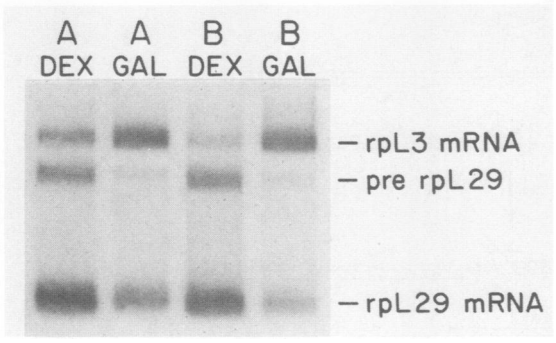


FIG. 4. Northern blot hybridization analysis of RNA from pGTCMΔ3′A and pGTCMΔ3′B transformants. Cells were grown in either glucose or galactose to a density of ca. 10^7 cells per ml. Total cellular RNA was extracted, and 3 μg of this RNA was electrophoresed, blotted to nitrocellulose, and hybridized to rpL3 and rpL29 single-stranded phage probes. Gel lanes labeled A correspond to RNA from the pGTCMΔ3′A transformant; those labeled B represent RNA from the pGTCMΔ3′B transformant.

Further characterization of strains with conditionally expressed *tcm1* genes. To examine in more detail the properties of the haploid strains bearing galactose-inducible rpL3 genes, we first measured their growth rate. Both pGTCMΔ3′A and Δ3′B haploid derivatives exhibited normal doubling times of ca. 3 h when galactose was provided as carbon source (data not shown). The pGTCMΔ3′A strain had a doubling time of 7.5 h on glucose, whereas the pGTCMΔ3′B strain, with the rpL3 promoter that is apparently completely inactive on glucose, did in fact grow on this carbon source, although its doubling time was about 12 to 18 h.

Since the assay of β-galactosidase directed by these two hybrid promoters suggested a reduction in transcriptional activity of several hundredfold on glucose (Fig. 2), we did not expect that either transformed strain would grow on glucose. Therefore, we examined the amount of *tcm1* mRNA present in the transformants. Figure 4 shows a Northern blot analysis of the steady-state levels of *tcm1* mRNA and another ribosomal protein transcript encoding rpL29 (2). Each lane of the gel was loaded with the same amount of total cellular RNA. It is clearly evident that rpL3 mRNA was depleted in cells propagated on glucose. Densitometric quantitation of the autoradiograph revealed that the pGTCMΔ3′A transformant contained only about 20% as much rpL3 mRNA and the pGTCMΔ3′B transformant had less than 10% as much of this mRNA when grown on glucose as compared to galactose. The abundance of the rpL3 mRNA is normalized to the density of the rpL29 band in the same lane of the gel. Essentially identical results were found with the yeast actin gene (16) as a normalization probe (data not shown). Thus, this analysis confirmed that the altered *tcm1* genes do in fact lead to a reduced abundance of rpL3 mRNA under the appropriate conditions. Note also that the rpL29 probe yielded a stronger hybridization signal with RNA from glucose-grown cells as compared to galactose grown cells. Because glucose-grown cells are limited in their ability to synthesize rpL3, they must accumulate fewer ribosomes. Consequently, glucose-grown cells must contain less RNA per cell, since ribosomal RNA accounts for 80 to 85% of total cellular RNA. By loading equal amounts of RNA in each lane of the gel, we actually analyzed the contents of more cells in the glucose-grown samples than in the galactose-grown samples.

Interestingly, the abundance of the rpL3 transcript in the transformed strains growing on glucose, 10 to 20% of normal, was not as low as predicted, based on measurements of the activity of the hybrid promoters when they were linked to the *lacZ* gene in plasmids pGTCM-Z2 and pGTCM-Z3. These measurements suggested a reduction in transcription on glucose to less than 0.5% of that on galactose. Indeed, in cells carrying pGTCM-Z2 and pGTCM-Z3, we were unable to detect an rpL3-β-GAL hybrid mRNA by Northern analysis of glucose-grown cells, whereas such a transcript was detected readily in RNA from galactose-grown cells (data not shown). Of course, the prediction of transcriptional activity assumes that the hybrid promoters act the same

whether contained within plasmids or within the yeast cell genome. Experiments are in progress to determine the rates of transcription of the hybrid rpL3 genes in their chromosomal context, but if we assume that their activity was in fact less than 0.5% of normal, the Northern analysis implies that the turnover rate of the rpL3 mRNA was slowed in glucose-grown cells to achieve the steady-state abundance we observed. Possibly, reduced turnover is related to the requirement of rpL3 for ribosome assembly, an effect which would not be expected for the rpL3-β-GAL transcript (see below).

Additional support for the possibility of a reduced turnover rate is derived from an analysis of the decay of preexisting mRNA when transcription of *tcm1* is halted upon shifting cells from galactose to glucose. Figure 5A shows a Northern analysis of RNA extracted at various times after pGTCMΔ3′B cells were harvested from galactose and suspended in glucose. The relative abundance of rpL3 mRNA was determined by densitometry (normalized to the rpL29 transcript) and plotted on a semilogarithmic scale (Fig. 5B). The initial slope of the graph shows that half of the rpL3 mRNA disappeared in the first 12 min after the shift. This decay is consistent with the 13-min half-life measured previously in wild-type cells by in vivo labeling techniques (12), and it implies that transcription of *tcm1* ceased immediately upon transfer to glucose. However, quantitation at later times suggests that the half-life of rpL3 mRNA is progressively increased, as the half-life measured from the semilog plot was ca. 23 min between 15 and 60 min after the shift and perhaps as long as 45 min during the following hour. More accurate in vivo labeling measurements are in progress to confirm this reduced rate of decay. Its possible significance is discussed below.

Finally, it should be noted that the *GAL10* activation sequence is responsible for very efficient transcription, with 0.25 to 1% of total mRNA being accounted for by GAL transcripts. Estimates of the abundance of ribosomal protein mRNAs have been placed at 0.1 to 0.2% of total mRNA (6), although this may be an underestimate (see reference 12). We found that rpL3 mRNA was about twice as abundant in the transformed strain grown on galactose as it was in a wild-type strain (Fig. 5A), indicating that the *GAL10* activation sequence is probably just as efficient upstream of *tcm1* as it is upstream of the *GAL10* gene.

Construction of a yeast strain with a conditionally expressed *cyh2* gene. Armed with the information gained from the construction of a galactose-inducible *tcm1* gene, we applied the same methodology to *cyh2*, a mutant allele of ribosomal protein L29 conferring resistance to cycloheximide (2, 13). Like *tcm1*, *cyh2* is present in only a single copy in the *S. cerevisiae* genome, but unlike *tcm1*, *cyh2* contains an intervening sequence (2). Cells transformed with additional copies of *cyh2* accumulate unspliced rpL29 precursor transcript, suggesting that processing of this mRNA may be modulated to control the synthesis of rpL29 (J. R. Warner, G. Mitra, W. F. Schwindinger, M. Studeny, and H. M. Fried, submitted for publication). Thus, we wished to obtain a strain which could be made to underproduce rpL29 mRNA for future analysis of the role of mRNA processing in regulation of rpL29 synthesis.

To obtain a yeast strain with a conditionally expressed *cyh2* gene, we inserted a restriction enzyme fragment of *cyh2* into pJT24, placing the *GAL10* activation sequence at about −160 relative to the start site of transcription of *cyh2* (see above). The distal end of the fragment did not join the *lacZ* moiety of pJT24 to the *cyh2* translational reading frame.

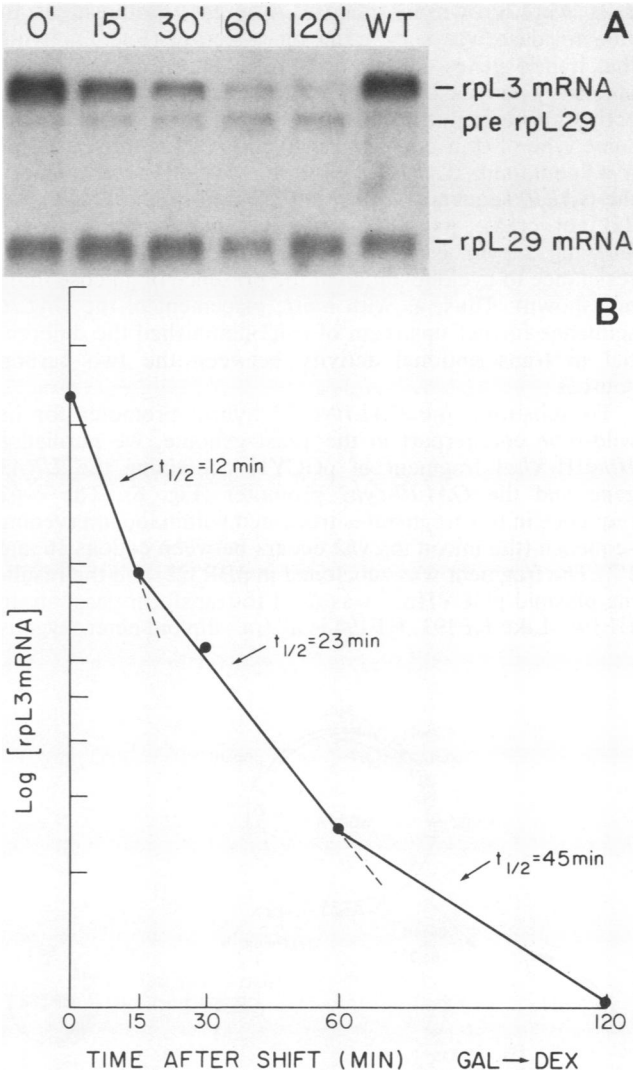


FIG. 5. Northern blot hybridization analysis of RNA extracted from pGTCMΔ3′ transformed cells at various times after a shift from galactose to glucose. (A) Cells were grown in galactose at 30°C to a density of about 10^7/ml, centrifuged for 2 min at 25°C and 4,000 rpm, and resuspended in their original volume with glucose-containing media (prewarmed to 30°C). At the indicated times, cells were withdrawn, frozen, and extracted for RNA. The gel lane marked WT contained RNA from a wild-type strain grown on galactose. (B) Semilog plot of the relative intensity of the rpL3 band (normalized to the rpL29 band) as a function of time after shift from galactose to glucose (GAL → DEX). $t_{1/2}$, Half-life of mRNA.

Nevertheless, the resulting plasmid pGCYH, when introduced into yeast cells, permitted growth in the presence of cycloheximide (5 μg/ml) and galactose, but no growth was observed with cycloheximide (1 μg/ml) plus glucose. Thus, the *GAL10* fusion to *cyh2* at −160 resulted in galactose-inducible expression of *cyh2*.

To get another idea of the relative levels of activity of the *GAL10-cyh2* promoter, we altered pGCYH to bring the *lacZ* coding sequence into the reading frame of rpL29 by cleaving at a site within amino acid codon no. 111 of *cyh2* and at the *Bam*HI site at the amino-terminal end of the *lacZ* segment (Fig. 6). Treatment with S1 nuclease, followed by recircularization, produced plasmid pGCYH-Z1. In yeast cells, pGCYH-Z1 did not produce detectable β-galactosidase when

cells were grown in glucose, whereas synthesis of β-galactosidase was substantial on galactose (Fig. 2). (Note that hybrid genes containing a yeast intron are processed efficiently in yeast [25].) Thus, placement of the *GAL10* activation sequence at position −160 in *cyh2* resulted in the same type of transcriptional regulation as that observed for *tcm1* containing *GAL10* at −138. In addition, we also placed the *GAL10* sequence at position −240 in *cyh2*; although we did not create its *lacZ* derivative, we found that cells carrying a *cyh2* gene with this promoter exhibited weak resistance to cycloheximide in the presence of glucose (data not shown). Thus, as with *tcm1*, placement of the *GAL10* sequence further upstream of *cyh2* diminished the differential in transcriptional activity between the two carbon sources.

To substitute the *GAL10-cyh2* hybrid promoter for its wild-type counterpart in the yeast genome, we purified a *Hin*dIII-*Xho*I fragment of pGCYH containing the *URA3* gene and the *GAL10-cyh2* promoter (Fig. 6). The *cyh2* sequence in this fragment is truncated within the intervening sequence (the intron in *cyh2* occurs between codons 16 and 17). The fragment was subcloned in pBR322, and the resulting plasmid pGCYHΔ3′ was used to transform yeast strain HF194. Like HF193, HF194 is a Ura⁻ diploid heterozygous

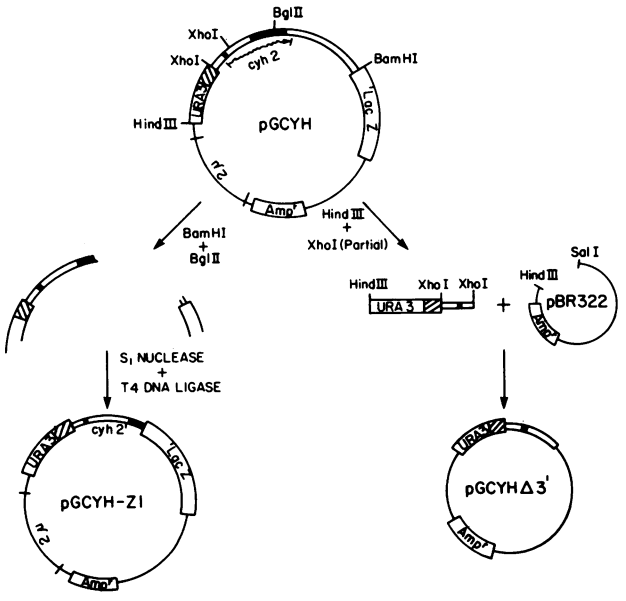


FIG. 6. Summary of plasmids used to obtain galactose-mediated regulation of *cyh2*. Plasmid pGCYH (top of figure) consists of the cloning vector pJT24, into which has been inserted a 2-kilobase *Tha*I restriction fragment of *cyh2*. The upstream end of this fragment, which adjoins the *GAL10* activation sequence (cross-hatched box), is at −160 relative to the transcriptional start site of *cyh2*. The downstream end of the fragment lies about 800 bp beyond the transcription termination site of *cyh2*. The two filled-in boxes in *cyh2* represent the two exons of this gene; the open box between them represents the intron. At the left is a diagram of the enzymology used to bring the *lacZ* moiety of pGCYH into the translational reading frame of *cyh2*, producing plasmid pGYCH-Z1, in which *lacZ* is joined to amino acid no. 111 of rpL29 (the product of *cyh2*). At the right is a diagram of the subcloning of a portion of pGCYH into pBR322. The subcloned fragment, in pGCYHΔ3′, contains the *URA3* gene and the *GAL10* activation sequence joined to *cyh2* at −160. The *cyh2* segment is truncated within the intron. Plasmid pGCYHΔ3′ was used to replace the promoter of *cyh2* in the yeast genome with its *GAL10*-regulated derivative (see the text).

for a drug resistance gene (*cyh2* in this case). Its use permitted initial identification of transformants arising from recombination of pGCYHΔ3′ at the *cyh2* locus. One such transformant was sporulated; all of the Ura⁺ spores grew normally on galactose but were completely nonviable on glucose. This phenotype is exactly the one expected if the *GAL10-cyh2* promoter has been joined to the rpL29 coding sequence in the yeast genome.

DISCUSSION

Ribosomal proteins are examples of macromolecules which are often called housekeeping proteins; that is, they are required by all cells at all times. Because of the essential role they fulfill, selection of mutations in ribosomal proteins has usually been limited to obtaining variants producing resistance to inhibitors of protein synthesis. In this report we describe a reverse genetic approach to obtaining a second category of ribosomal protein gene variants, namely, conditional lethal mutants. We began with two cloned yeast ribosomal protein genes and modified their promoters to a form requiring the presence of an inducer (galactose) for transcription. In the presence of galactose, the genes are expressed at a normal level, yielding wild-type growth rates. In the absence of the inducer, however, the genes are expressed either weakly or essentially not at all, resulting in slower or nonexistent growth. This galactose-dependent expression of the ribosomal protein genes was obtained by placing, at a distance of between 90 and 230 nucleotides upstream of their transcription start sites, an activation sequence derived from the *GAL10* gene. This sequence mediates normally the galactose-induced expression of the *GAL1* and *GAL10* genes, probably through interaction with the *GAL4* protein (5, 10, 19). The hybrid ribosomal protein genes described here require the *GAL4* protein for expression as well.

Construction of the galactose-regulated ribosomal protein genes was facilitated by the prior placement of the 365-bp *GAL10* activation sequence upstream of *CYC1*, which itself was joined to the *E. coli lacZ* gene, and by the results of Guarente and co-workers, which demonstrated that the *GAL10* sequence is sufficient to confer galactose inducibility upon *CYC1* (5). We modified the *GAL10-CYC1-lacZ* gene by replacing the *CYC1* segment with several unique restriction enzyme sites to produce a generalized cloning vector for placing any gene under galactose regulation. Whereas the two ribosomal protein genes we chose to modify, *tcm1* and *cyh2*, themselves confer easily identifiable phenotypes (resistance to inhibitors), plasmid pJT24 is especially useful for obtaining galactose-regulated expression of the majority of housekeeping genes which do not confer a useful phenotype, since the expression of β-galactosidase is substituted for the normal gene product. *tcm1* and *cyh2* were also selected because they occur only once in the *S. cerevisiae* genome (1, 2), thus simplifying their replacement with the *GAL10* derivatives. However, given that methods are available for deleting any cloned yeast gene from the genome (20, 22), in principle, by prior deletion of one of its two loci, even those ribosomal proteins encoded by two genes could be subjected to galactose regulation.

Plasmid pJT24 is useful also because it permits placement of the *GAL10* activation sequence at any distance upstream of the gene of interest. As we have shown, it is possible to achieve somewhat of a range in transcriptional activity by locating the activation sequence at various distances from the start site of transcription. This effect is probably the result of progressive removal of the sequences which deter-

mine the efficiency of transcription of the target gene, thus making it more dependent upon the *GAL10* activation sequence for expression. For housekeeping proteins which interact with a number of other proteins in a multimeric complex, the ability to reduce but not eliminate transcription of their genes should permit examination of compensating mechanisms which act to maintain a balanced synthesis of those proteins. For example, we have shown recently that yeast cells which overproduce *tcm1* mRNA do not increase the rate of synthesis of rpL3, suggesting a translational compensating mechanism which assures a stoichiometric synthesis of that protein (17). An identical phenomenon has been found for cells overproducing *cyh2* mRNA (J. Warner et al., submitted for publication). In this report, we describe cells which underproduce *tcm1* mRNA by virtue of its new promoter being only weakly active in the absence of galactose. However, assuming that the activity of the chromosomally located *GAL10-tcm1* promoter is the same as it is in the plasmid in which it was first constructed, there is a large discrepancy between the inferred reduction in transcription of *tcm1* and the actual steady-state abundance of its mRNA. In this case, faced with a depletion of a single ribosomal protein mRNA, perhaps yeast cells enhance translational efficiency so as to continue synthesis of the essential protein. Enhanced translation would then lead to a prolonged lifetime of the mRNA and an elevated steady-state abundance. An extended lifetime is suggested also by the quantitation of rpL3 mRNA at various times after shifting cells from galactose to glucose. Upon immediate cessation of transcription, the preexisting rpL3 mRNA decayed with a half-life of 12 min, but examination of later time points suggested a half-life of 23 to 45 min. Experiments are in progress to measure the translational efficiency of rpL3 mRNA at the various times after carbon source shift.

The hybrid ribosomal protein genes we have constructed will be useful for studying other aspects of the regulation of ribosome biosynthesis besides translational phenomena. For instance, we have found that cells transformed with a multicopy plasmid containing *cyh2* accumulate unspliced rpL29 precursor RNA (Warner et al., submitted for publication). Either this limited processing is a regulatory response preventing oversynthesis of rpL29, a reflection of an inherently slow rate of rpL29 mRNA processing, or possibly an artifact of the plasmid. It will be of interest to determine whether introduction of the multicopy plasmid into cells with reduced transcriptional activity of the chromosomal allele of *cyh2* results in an increase in the extent of processing of the plasmid-derived precursor transcripts.

Finally, the conditional lethal strains we have described demonstrate that ribosomal proteins L3 and L29 are essential for cell viability (strictly speaking, the pGTCMΔ3'B strain retains some ability to synthesize rpL3 and thus is not completely inviable in the absence of galactose). Furthermore, these strains will make it feasible to isolate additional ribosomal protein mutations affecting the assembly of the proteins, their function within the ribosome, or perhaps their cytoplasmic-nuclear transport. Mutagenized *tcm1* and *cyh2* plasmids can be introduced into the conditional lethal strains. Plasmids unable to restore growth of the recipient cell under the nonpermissive condition (glucose) would be candidates for mutants defective in some aspect of ribosome biogenesis.

ACKNOWLEDGMENTS

This work was supported by National Science Foundation grant PCM-8215576, by Public Health Service general research support award 5-S01-FR-05406 from the National Institutes of Health, and by American Cancer Society institutional grant IN-15X. J.T. was supported by Public Health Service grant GM23549 from the National Institutes of Health.

We thank Jacqueline Bromberg and Mark Underwood for stimulating discussions and Leonard Guarente and Stephan Johnston for valuable advice and for providing strains and plasmids.

LITERATURE CITED

1. **Fried, H. M., and J. R. Warner.** 1981. Cloning of the yeast gene for trichodermin resistance and ribosomal protein L3. Proc. Natl. Acad. Sci. U.S.A. **78**:238–242.
2. **Fried, H. M., and J. R. Warner.** 1982. Molecular cloning and analysis of yeast gene for cycloheximide resistance and ribosomal protein L29. Nucleic Acids Res. **10**:3133–3148.
3. **Fried, H. M., and J. R. Warner.** 1984. Organization and expression of eukaryotic ribosomal protein genes, p. 169–192. *In* G. S. Stein and J. L. Stein (ed.), Recombinant DNA and cell proliferation. Academic Press, Inc., New York.
4. **Grant, P. G., D. Schindler, and J. E. Davies.** 1976. Mapping of trichodermin resistance in *Saccharomyces cerevisae*: a genetic locus for a component of the 60S ribosomal subunit. Genetics **83**:667–682.
5. **Guarente, L., R. R. Yocum, and P. Gifford.** 1982. A *GAL10-CYC1* hybrid yeast promoter identifies the *GAL4* regulatory region as an upstream site. Proc. Natl. Acad. Sci. U.S.A. **79**:7410–7414.
6. **Hereford, L. M., and M. Rosbash.** 1977. Regulation of a set of abundant mRNA sequences. Cell **10**:463–467.
7. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. U.S.A. **75**:1929–1933.
8. **Hopper, J. E., J. R. Broach, and L. B. Rowe.** 1978. Regulation of the galactose pathway in *Saccharomyces cerevisae*: induction of uridyl transferase mRNA and dependency on *GAL4* gene function. Proc. Natl. Acad. Sci. U.S.A. **75**:2878–2882.
9. **Hu, N., and J. Messing.** 1982. The making of strand-specific M13 probes. Gene **17**:271–277.
10. **Johnston, M., and R. W. Davis.** 1984. Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:1440–1448.
11. **Käufer, N. F., H. M. Fried, W. F. Schwindinger, M. Jasin, and J. R. Warner.** 1983. Cycloheximide resistance in yeast: the gene and its protein. Nucleic Acids Res. **11**:3123–3135.
12. **Kim, C. H., and J. R. Warner.** 1983. Messenger RNA for ribosomal proteins in yeast. J. Mol. Biol. **165**:79–89.
13. **McLaughlin, C. S.** 1974. Yeast ribosomes: genetics, p. 815–827. *In* M. Nomura, A. Tissières, and P. Lengyel (ed.), Ribosomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either double-digest restriction fragments. Gene **19**:269–276.
15. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Ng, R., and J. Abelson.** 1980. Isolation and sequence of the gene for actin in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **77**:3912–3916.
17. **Pearson, N. J., H. M. Fried, and J. R. Warner.** 1982. Yeast use translational control to compensate for extra copies of a ribosomal protein gene. Cell **29**:347–355.
18. **Penman, S., A. Fulton, D. Capco, A. Ben Ze'ev, S. Wittlesberger, and C. F. Tse.** 1981. Cytoplasmic and nuclear architecture in cells and tissue: form, functions, and mode of assembly. Cold Spring Harbor Symp. Quant. Biol. **46**:1013–1028.
19. **Perlman, D., and J. E. Hopper.** 1979. Constitutive synthesis of the *GAL4* protein, a galactose pathway regulator in *Saccharomyces cerevisiae*. Cell **16**:89–95.
20. **Rothstein, R. J.** 1983. One step gene disruption in yeast. p. 202–211. Methods Enzymol. **101**:202–211.
21. **Schultz, L. D., and J. D. Friesen.** 1983. Nucleotide sequence of the *tcm1* gene (ribosomal protein L3) of *Saccharomyces cerevisiae*. J. Bacteriol. **155**:8–14.
22. **Shortle, D., J. E. Haber, and D. Botstein.** 1982. Lethal disruption

of the yeast actin gene by integrative DNA transformation. Science **217**:371–373.

23. **St. John, T., and R. Davis.** 1979. Isolation of galactose inducible DNA sequence from *Saccharomyces cerevisiae* by differential plaque filter hybridization. Cell **16**:443–452.
24. **St. John, T. P., and R. W. Davis.** 1981. The organization and transcription of the galactose gene cluster of *Saccharomyces*. J. Mol. Biol. **152**:285–315.
25. **Teem, J. L., and M. Rosbash.** 1983. Expression of a β-galactosidase gene containing the ribosomal protein 51 intron is sensitive to the *rna2* mutation of yeast. Proc. Natl. Acad. Sci. U.S.A. **50**:4403–4407.
26. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
27. **Warner, J. R.** 1982. The yeast ribosome: structure, function, and synthesis, p. 529–560. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast saccharomyces. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Warner, J. R., and C. Gorenstein.** 1977. The synthesis of eukaryotic ribosomal proteins in vitro. Cell **11**:201–212.
29. **Warner, J. R., R. J. Tushinski, and P. J. Wejksnora.** 1980. Coordination of RNA and proteins in eucaryotic ribosome production, p. 889–902. *In* G. Chambliss, R. G. Craven, J. Davies, K. Davis, L. Kahan, and M. Nomura (ed.), Ribosomes: structure, function, and genetics. University Park Press, Baltimore.